الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
Garden of Knowledge and Virtue

IIUM KUANTAN
DEPARTMENT OF CHEMISTRY

EFFECT OF DIFFERENT EXTRACTION METHODS ON VITAMIN B12 FROM EDIBLE GREEN SEAWEED, *ULVA LACTUCA* USING RESPONSE SURFACE METHODOLOGY

FATIN SHAZWANI RUSLAN, DENY SUSANTI, NORMAWATY MOHAMMAD NOR, MUHAMAD TAHER, NURUL IMAN AMINUDDIN

RESEARCH INTRODUCTION

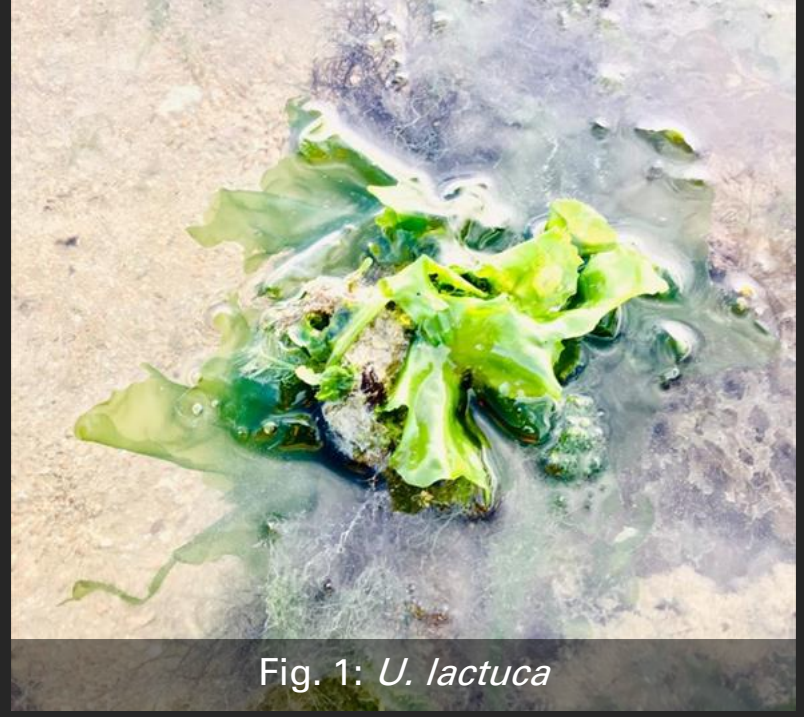
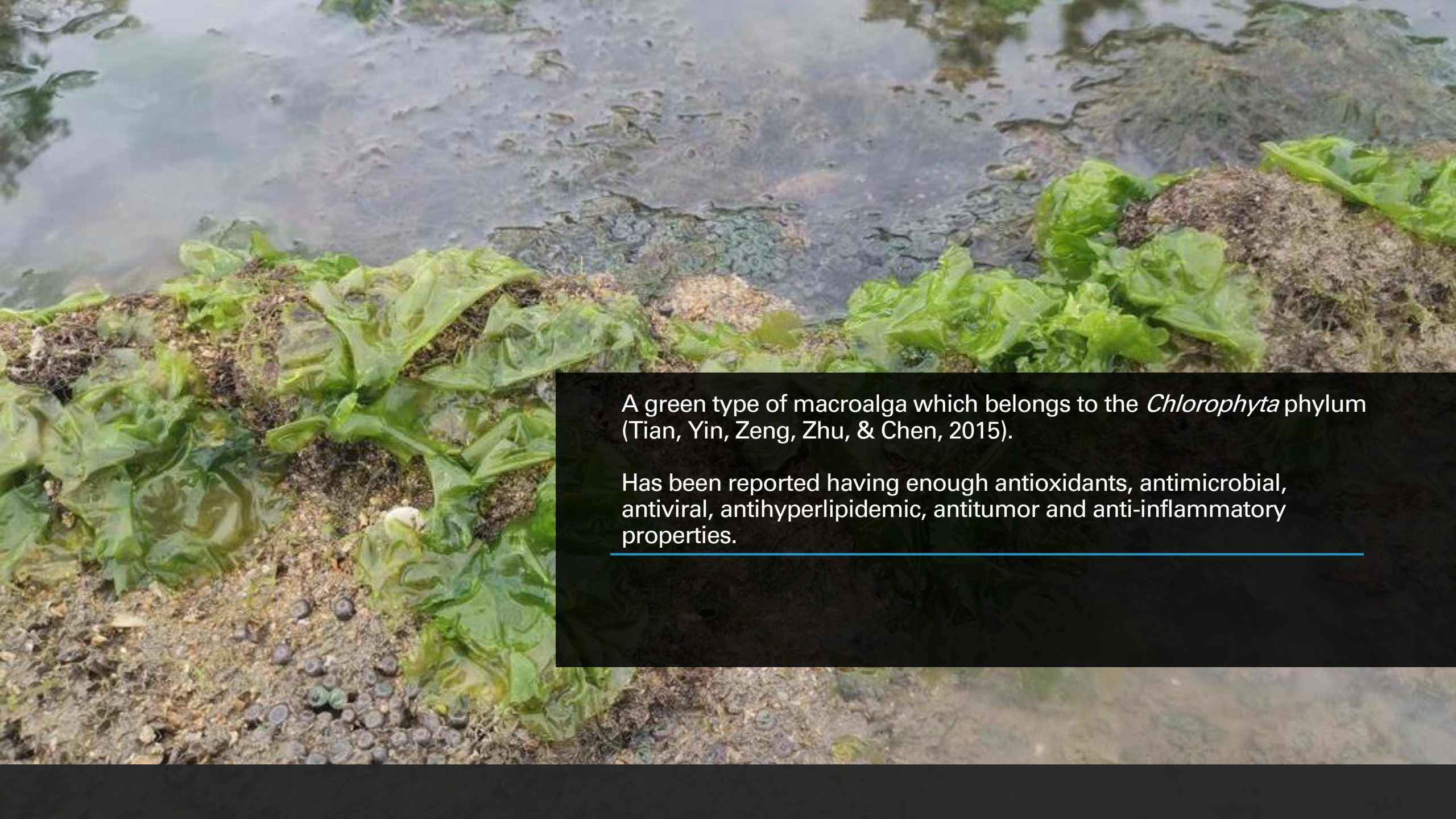


Fig. 1: *U. lactuca*

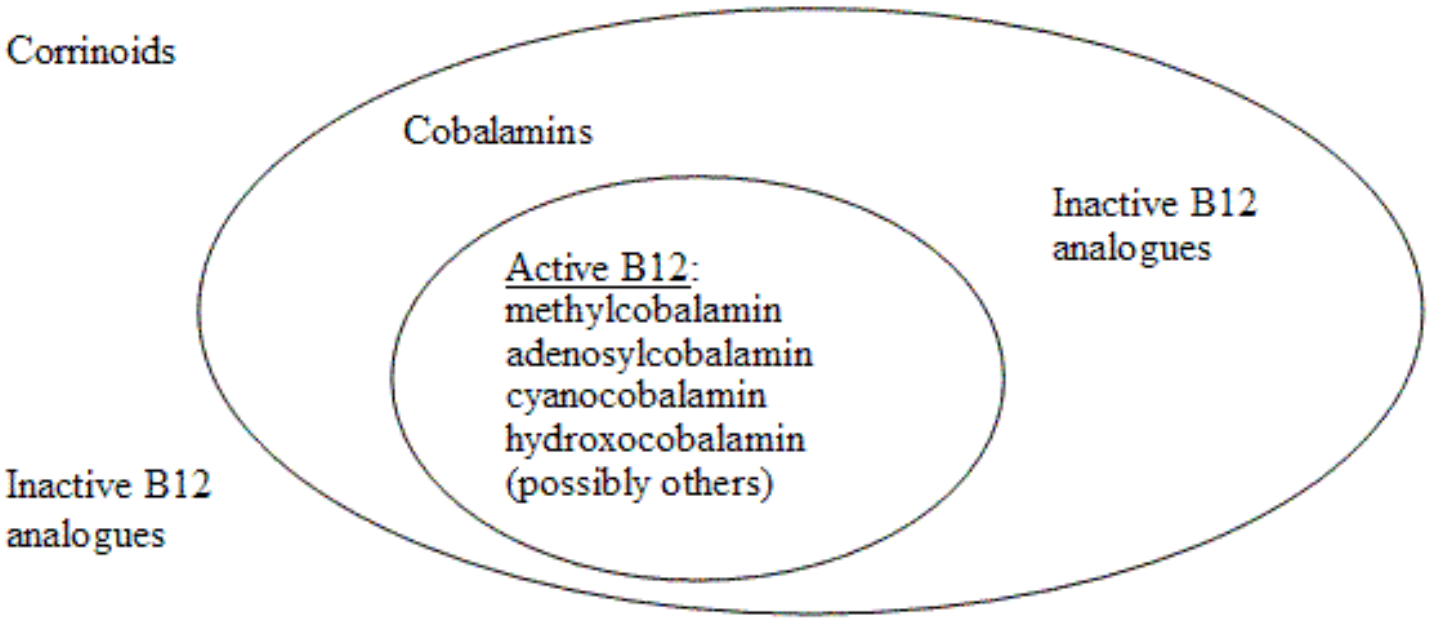
Fig. 2: Vitamin B12

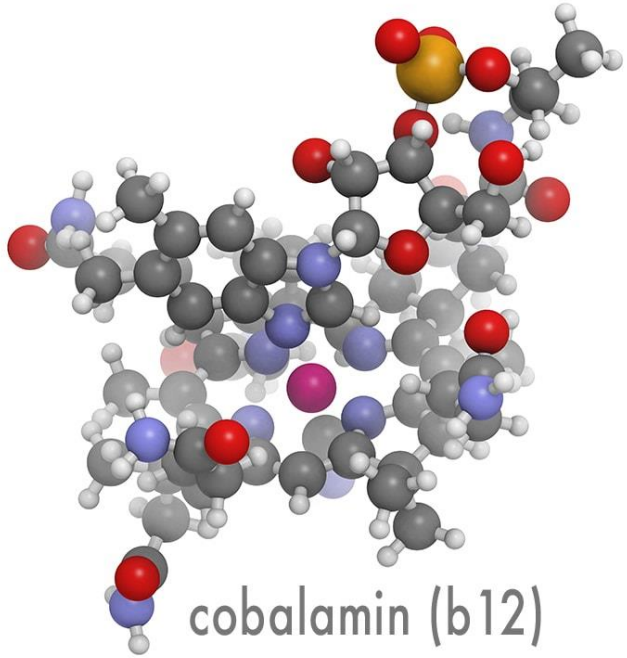
Fig. 3: Extraction of vitamin B12



A green type of macroalga which belongs to the *Chlorophyta* phylum (Tian, Yin, Zeng, Zhu, & Chen, 2015).

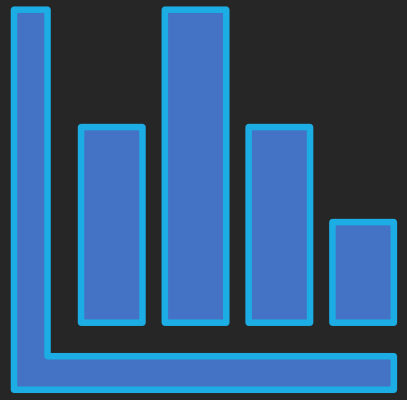
Has been reported having enough antioxidants, antimicrobial, antiviral, antihyperlipidemic, antitumor and anti-inflammatory properties.





- Strange sensations, numbness, or tingling in the hands, legs
- Difficulty walking (staggering, balance problems)
- Anemia
- Difficulty thinking and reasoning (cognitive difficulties), or memory loss
- Fatigue

VITAMIN B12 DEFICIENCY



METHODOLOGY

OVERVIEW OF THE PROJECT

- Sample collection, drying and preparation
- Designing screening experimental design by 2-Level Factorial
- Sample extraction using different extraction methods and parameters
- Purification of vitamin B12 crude extract
- Qualitative and quantitative analysis by HPLC
- Statistical analysis
- Optimisation by Central Composite Design (CCD)




- *U. lactuca* were let to dry accordingly by freeze-, sun-, oven-, and air-drying techniques.

SAMPLE COLLECTION, DRYING AND PREPARATION

Run	Solvent:solvent ratio (MeOH:H2O)	pH	Solute:solvent ratio (g/mL)	Conc. of vitamin B12 (mg/mL) *
1	75:25	3	3:60	
2	50:50	4	3:75	
3	25:75	3	3:90	
4	75:25	5	3:60	
5	25:75	5	3:60	
6	25:75	3	3:90	
7	50:50	4	3:75	
8	75:25	3	3:90	
9	50:50	4	3:75	
10	25:75	5	3:90	
11	75:25	5	3:90	
12	75:25	5	3:90	
13	25:75	3	3:60	
14	75:25	3	3:90	
15	75:25	3	3:60	
16	25:75	5	3:90	
17	75:25	5	3:60	
18	50:50	4	3:75	
19	50:50	4	3:75	
20	25:75	3	3:60	
21	25:75	5	3:60	

- 25: 75 – 75:25 % Solvent: solvent ratio (A)
- pH 3-5 (B)
- 3: 60 – 3: 90 g/mL (C)

EXPERIMENTAL DESIGN BY TWO-LEVEL FACTORIAL

Ultrasonic-assisted extraction	▪ Dried sample was suspended in the solvent mixture, extracted at 20 kHz, for 20 minutes at 65 °C.
Orbital shaker	▪ Dried sample was shaken in the orbital shaker at 200 rpm for 30 minutes.
Boiling	▪ Dried sample was boiled for 20 minutes at 65 °C.

EXTRACTION OF DRIED *U. LACTUCA* SAMPLES

RESULTS AND DISCUSSION

1) QUALITATIVE AND QUANTITATIVE ANALYSIS OF VITAMIN B12 BY HPLC

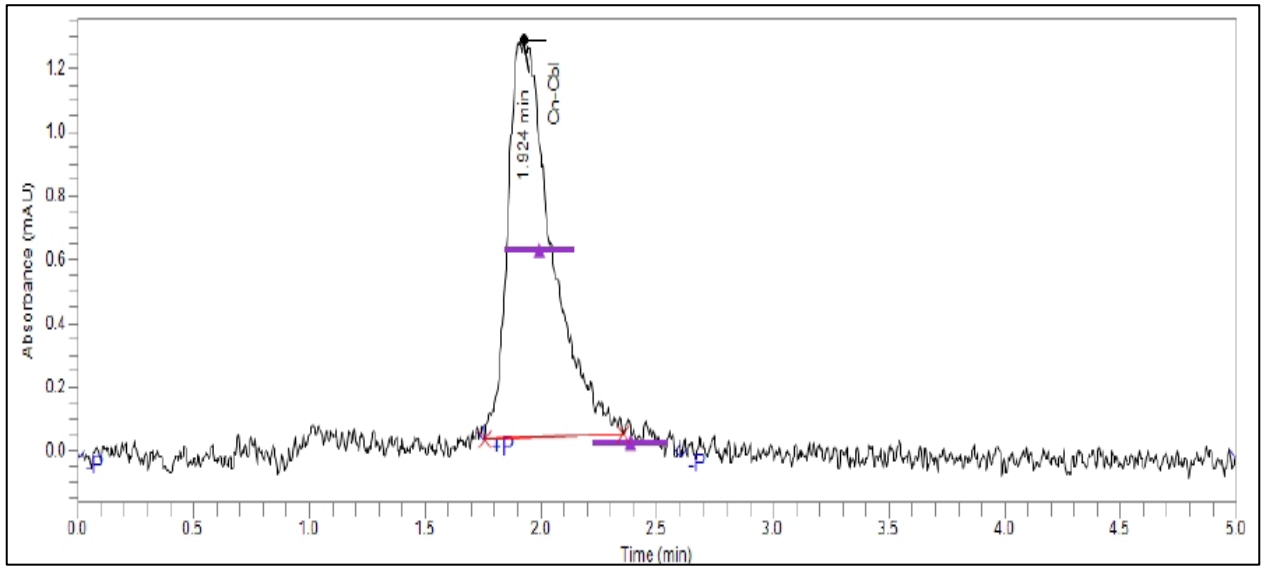


Fig. 4: The HPLC chromatogram of one of the ADB sample, contained Cyanocobalamin, with the retention time was at 1.924 min. The other peaks represented other vitamin B12 compounds were not appeared.

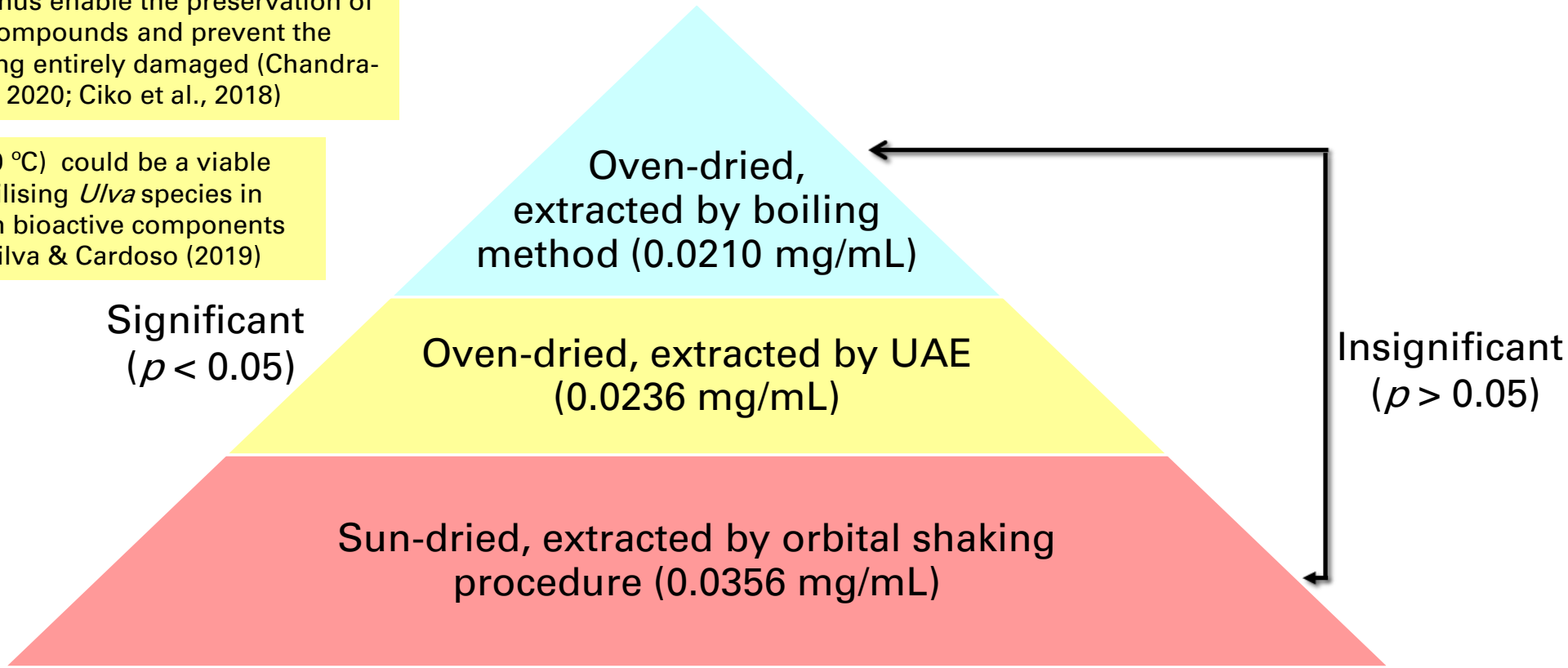
Table 1: Retention time of the standards, obtained from HPLC

Standards	Retention time (min)
Cn-Cbl	1.9
OH-Cbl	2.3
Adl-Cbl	2.7
Me-Cbl	3.1

2) SCREENING OF EXTRACTION METHODS AND DRYING CONDITIONS

The properties of UAE method which operate at low temperature thus enable the preservation of thermolabile compounds and prevent the structure from being entirely damaged (Chandra-Hioe et al., 2020; Ciko et al., 2018)

Oven-drying (40 °C) could be a viable option for stabilising *Ulva* species in extracting certain bioactive components Silva, Abreu, Silva & Cardoso (2019)

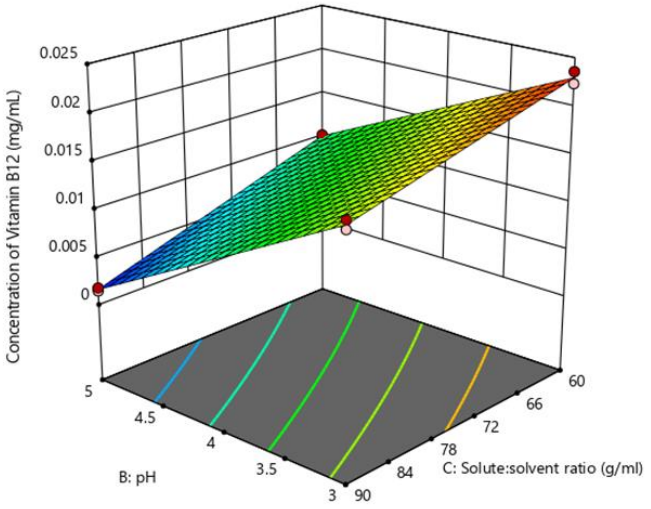


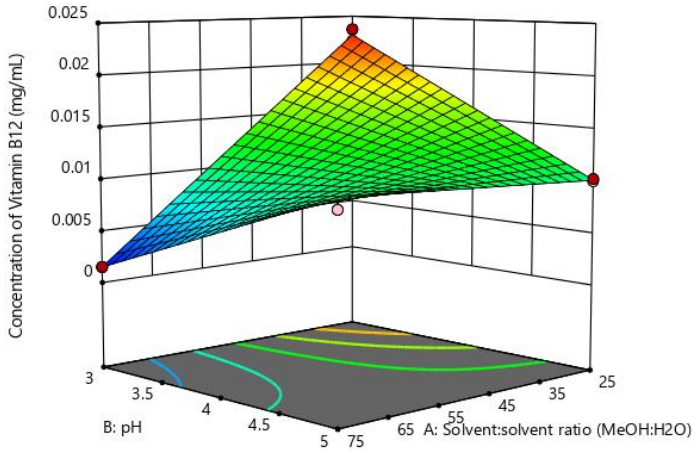
3) SCREENING FOR SIGNIFICANT FACTORS USING 2-LEVEL FACTORIAL DESIGN

Table 2: ANOVA analysis for the concentration of CN-Cbl from oven-dried *U. lactuca* using UAE method

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0008	7	0.0001	76.53	< 0.0001	significant
A-Solvent:solvent ratio (MeOH:H2O)	0.0000	1	0.0000	10.53	0.0070	
B-pH	0.0001	1	0.0001	49.69	< 0.0001	
C-Solute:solvent ratio	2.448E-06	1	2.448E-06	1.69	0.2180	
AB	0.0004	1	0.0004	261.72	< 0.0001	
AC	0.0003	1	0.0003	184.21	< 0.0001	
BC	0.0000	1	0.0000	20.66	0.0007	
ABC	0.0000	1	0.0000	7.22	0.0198	
Curvature	0.0001	1	0.0001	60.40	< 0.0001	

C possessed a less remarkable statistical relevance in the extraction procedure (Azwanida, 2015).


Concentration of Vitamin B12 (mg/mL)
0.025
0.02
0.015
0.01
0.005
0
5
4.5
4
3.5
3
B: pH
60
66
72
78
84
90
C: Solute:solvent ratio (g/ml)


Concentration of Vitamin B12 (mg/mL)
0.025
0.02
0.015
0.01
0.005
0
3
3.5
4
4.5
5
B: pH
25
35
45
55
65
75
A: Solvent:solvent ratio (MeOH:H2O)

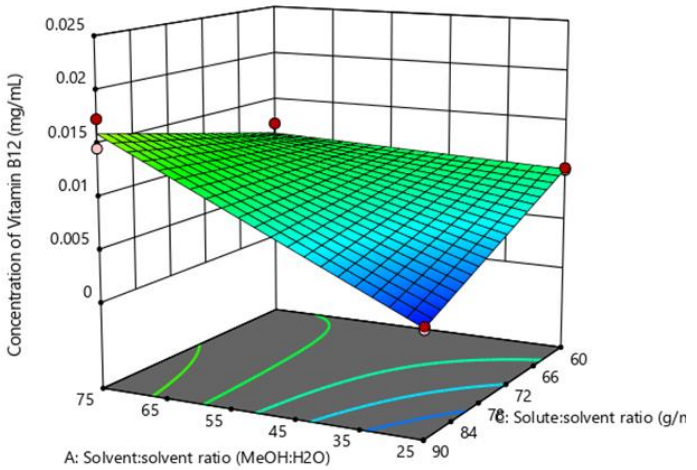

Concentration of Vitamin B12 (mg/mL)
0.025
0.02
0.015
0.01
0.005
0
75
65
55
45
35
25
A: Solvent:solvent ratio (MeOH:H2O)
60
66
72
78
84
90

Table 3: Optimised design layout of Central composite design for UAE of oven-dried sample

Run	Solvent:solvent ratio ($MeOH:H_2O$)	pH	Conc. of vitamin B12 (mg/mL)
1	0:100	3	0.0250
2	50:50	3	0.0084
3	0:100	5	0.0072
4	50:50	5	0.0055
5	0:100	4	0.0323
6	50:50	4	0.0189
7	25:75	3	0.0225
8	25:75	5	0.0192
9	25:75	4	0.0357
10	25:75	4	0.0355
11	25:75	4	0.0351
12	25:75	4	0.0358
13	25:75	4	0.0305

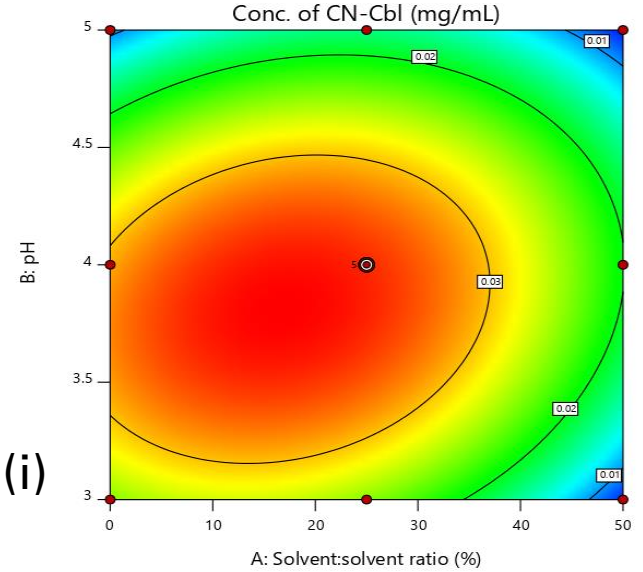
4) CN-Cbl yield optimisation of ODU using Central Composite Design

4) CN-Cbl yield optimisation of ODU using Central Composite Design

Table 3: ANOVA analysis for the concentration of CN-Cbl from oven-dried *U. lactuca* using UAE method

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0003	5	0.0003	49.26	< 0.0001	significant
A-Solvent:solvent ratio	0.0002	1	0.0002	27.14	0.0012	
B-pH	0.0001	1	0.0001	15.64	0.0055	
AB	0.0001	1	0.0001	9.06	0.0196	
A^2	0.0002	1	0.0002	37.27	0.0005	
B^2	0.0005	1	0.0005	85.86	< 0.0001	
Residual	0.0000	7	6.179E-07			
Lack of Fit	0.0000	3	7.511E-06	1.45	0.3538	not significant
Pure Error	0.0000	4	5.180E-06			
Cor Total	0.0016	12				

(i)



(ii)

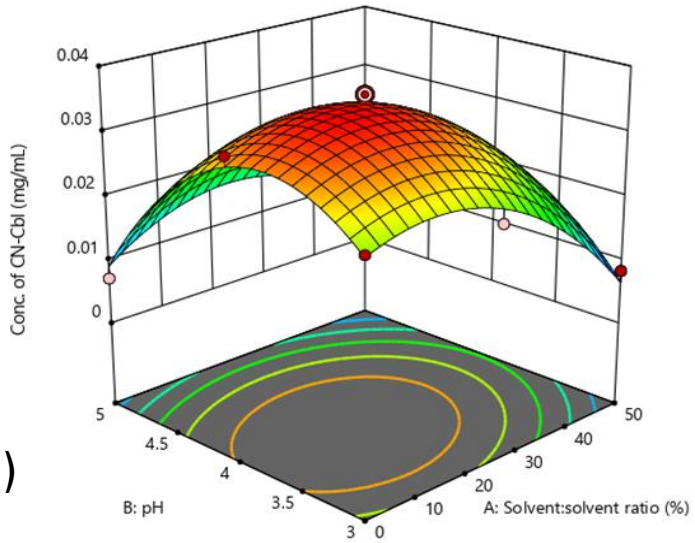
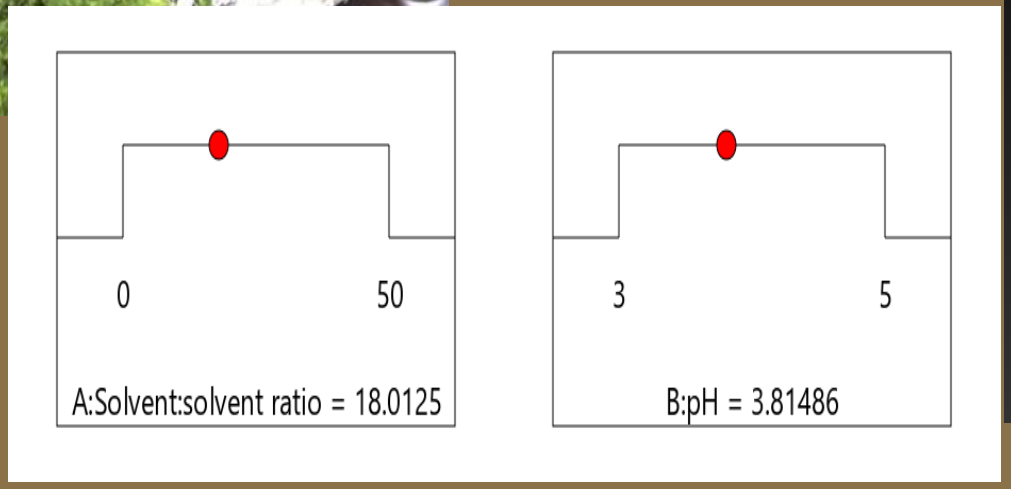


Fig 5: (i) AB interaction in contour plots
(ii) AB interaction in 3D surface



CONCLUSION


Thank
you